

INCIPIENT SEXUAL ISOLATION IN
DROSOPHILA PSEUDOOBSCURA BOGOTANA
AYALA & DOBZHANSKY
(DIPTERA: DROSOPHILIDAE)*

MOHAMED A. NOOR

Department of Ecology and Evolution, University of Chicago,
Chicago, Illinois 60637

Abstract. — Incipient sexual isolation was found between the subspecies *Drosophila pseudoobscura bogotana* (Ayala and Dobzhansky) and *D. p. pseudoobscura* (Frolova). Males took longer to initiate courtship with heterosubspecifics than consubspecifics. This divergence could represent an early step in the speciation of these taxa. The subspecies' pheromones do not appear to have diverged, so the difference is attributed to female behavior.

Key Words. — Insecta, *Drosophila pseudoobscura*, *Drosophila pseudoobscura bogotana*, mating behavior, sexual isolation, reproductive isolation, speciation

Behavioral characters in geographically isolated populations of a species can diverge over time. If they are separated long enough, their sexual behavior might differentiate to the extent that individuals of one population would not recognize individuals of the other population as potential mates. The two populations will then be different species.

Many *Drosophila* species have courtship patterns that prevent them from hybridizing with other species in nature (Brown 1965, Spieth 1951, Carson et al. 1989). Conspecific populations which are geographically isolated and diverging genetically provide ideal subjects for studying incipient sexual isolation. Does the divergence evolve rapidly, or are these behavioral changes gradual? Studies of the initial stages of ethological differentiation have been largely neglected; researchers have focused instead on estimating sexual isolation between sibling species pairs (Ryan & Wilczynski 1991). I present a test of incipient sexual isolation in two subspecies of *Drosophila*.

Drosophila pseudoobscura bogotana occurs at high elevations in the vicinity of Bogota, Columbia, nearly 2400 km from the main body of the species (Dobzhansky et al. 1963). It has differentiated genetically from the North American mainland *D. pseudoobscura pseudoobscura* Frolova, as evidenced by allozyme (Ayala & Dobzhansky 1974, Coyne & Felton 1977, Singh 1983) and DNA-sequence analyses (Schaeffer & Miller 1991); the latter estimated that they became geographically isolated approximately 150,000 years ago. Although there appears to be extensive gene flow among the North American populations, there is no evidence for gene exchange between the subspecies (Schaeffer & Miller 1992). Intersubspecific hybrid male offspring with *bogotana* mothers are sterile, while all other hybrid offspring are fertile.

There has been little research on the behavioral divergence between these subspecies. Prakash (1972) found that matings between consubspecifics were as fre-

* Author page charges partially offset by a grant from the C. P. Alexander Fund, PCES.

quent as matings between heterosubspecifics in chambers containing both subspecies. However, Singh (1983) noted that *bogotana* females preferred *pseudoobscura* males to their own males in such mixed cages.

Here, I report a new test of behavioral divergence of the subspecies *D. p. bogotana* and *D. p. pseudoobscura*. Since these subspecies are truly allopatric, any behavioral differences must have arisen by drift or correlated selection pressures imposed by their different environments rather than selection in sympatry to reduce hybridization (see Dobzhansky 1940).

MATERIALS AND METHODS

I observed the courtship of *bogotana* and *pseudoobscura* in several intrasubspecific and intersubspecific matings. Differences in length of time until courtship was initiated (courtship latency) and length of time between courtship initiation and copulation (courtship duration) were noted. The former shows the length of time the male took to identify the female as a potentially receptive mate. The latter shows how quickly the female allowed the male to copulate. These two characters represent differences in the specific behavioral components that can confer sexual isolation, such as differing acoustical, visual, olfactory, or tactile cues.

The *bogotana* strains used were an isofemale line from el Recreo (hereafter BOG1), originally captured in 1978, and line 0121.35 from the National Species Resource Center (hereafter BOG2), originally captured in 1960. They were chosen because they were hardy and showed no obvious symptoms of inbreeding depression, such as a consistent lack of intrastrain matings in 20 min observations. The USA1 stock was a mixture of four isofemale lines captured in the Gundlach-Bundschu Winery in the Sonoma Valley of California in 1988. The USA2 stock was an isofemale line collected in Flagstaff, Arizona in 1993.

Stocks were kept at 20° C on cornmeal/Karo syrup/yeast/agar food. Carbon dioxide was used for anesthetization. Virgin males and females were collected daily and kept individually in vials for 7 d to reach sexual maturity. Single male and female pairs were then placed without anesthesia in an 8 dram food-containing vial and observed for up to 20 min. Each combination was repeated 20 to 30 times. The time of first male courtship after introduction and time of copulation were recorded. First courtships were usually wing-vibrations (Brown 1963), but occasionally a male attempted to mount a female directly. The courtship duration was estimated as the time of first courting subtracted from the time of copulation. Any pairings in which the male or female was clearly weak or injured were excluded. The rare pairings (approximately 5%) in which copulation did not occur in the first 20 min were also excluded, since they likely resulted from nonvirginity or abnormalities in the specific flies. These unsuccessful pairings were randomly distributed among the crosses.

Because some strains had been in culture for several years, their behavior may have diverged from that of flies in nature. To correct for any selection within the strains, I compared interstrain crosses within subspecies to crosses with the other subspecies (see Tables 1 and 2). I performed Kruskal-Wallis and Mann-Whitney *U*-tests on courtship latency and courtship duration to assess differences in sexual behavior.

Table 1. Median courtship latencies (sec) and *P* value as estimated by two-tailed Mann-Whitney *U*-test.

Intrasubsp.		<i>n</i>	Median T	Intersubsp.		<i>n</i>	Median T	<i>P</i>
BOG1 Male ×	BOG2 Female	29	15	USA1 Female		28	32	0.0043
BOG2 Male ×	BOG1 Female	29	14	USA1 Female		22	19.5	0.5875
BOG1 Female ×	BOG2 Male	29	14	USA1 Male		29	39	0.0180
BOG2 Female ×	BOG1 Male	29	15	USA1 Male		22	15.5	0.5873
USA1 Male ×	USA2 Female	27	16	BOG1 Female		29	39	0.0136
USA1 Male ×	USA2 Female	27	16	BOG2 Female		22	15.5	0.8721
USA1 Female ×	USA2 Male	24	12.5	BOG1 Male		28	32	0.0001
USA1 Female ×	USA2 Male	24	12.5	BOG2 Male		22	19.5	0.0942

RESULTS

A Kruskal-Wallis test on courtship latencies showed significant heterogeneity among all crosses (two-tailed $H = 25.34$, 7 df, $P = 0.0007$), with the mean ranks of the intrasubspecific crosses all smaller than the mean ranks of the intersubspecific crosses (Table 1). The probability values from the Mann-Whitney *U*-tests were combined for two broad tests of significance, as described by Fisher (see Sokal & Rohlf 1969); the composite statistics showed significant tendencies for the male flies to court consubspecific females more quickly than heterosubspecific females (two-tailed $\chi^2 = 20.83$, 8 df, $P < 0.025$) and for the females to be courted more quickly by consubspecific males than heterosubspecific males (two-tailed $\chi^2 = 32.24$, 8 df, $P < 0.001$). These courtship latency data clearly indicate some subspecies discrimination.

The data for courtship duration were less conclusive. The Kruskal-Wallis test showed heterogeneity among all crosses ($H = 19.38$, 7 df, $P = 0.0062$), but intrasubspecific matings were not all faster than the intersubspecific matings (Table 2). However, no intersubspecific crosses were significantly faster than the corresponding intrasubspecific crosses.

DISCUSSION

These experiments demonstrate that *D. p. bogotana* has differentiated ethologically from the mainland *D. p. pseudoobscura*. Although the differences are minor compared to the level of sexual isolation between *D. pseudoobscura* and

Table 2. Median courtship durations (sec) and *P* value as estimated by two-tailed Mann-Whitney *U*-test.

Intrasubsp.		<i>n</i>	Median T	Intersubsp.		<i>n</i>	Median T	<i>P</i>
BOG1 Male ×	BOG2 Female	27	15	USA1 Female		22	13.5	0.9919
BOG2 Male ×	BOG1 Female	29	9	USA1 Female		17	45	0.0060
BOG1 Female ×	BOG2 Male	29	9	USA1 Male		28	36	0.0549
BOG2 Female ×	BOG1 Male	27	15	USA1 Male		20	7	0.3231
USA1 Male ×	USA2 Female	27	8	BOG1 Female		28	36	0.0046
USA1 Male ×	USA2 Female	27	8	BOG2 Female		20	7	0.4872
USA1 Female ×	USA2 Male	25	10	BOG1 Male		22	13.5	0.7133
USA1 Female ×	USA2 Male	25	10	BOG2 Male		17	45	0.0067

its sibling species, *D. persimilis* Dobzhansky and Epling (Tan 1946), they could represent an early stage in the development of stronger, and perhaps complete, isolation. The minor behavioral differences associated with the one-way hybrid male sterility in these taxa agree with previous observations that premating and postmating isolation may evolve in a correlated fashion among allopatric sibling species pairs (Coyne & Orr 1989).

Singh (1983) also noted that intersubspecific matings took longer than intrasubspecific matings in nonchoice experiments in these taxa, but he attributed the delays to female discrimination. The results of this study demonstrate that female subspecies discrimination may play little or no part in the observed mating delays.

The specific components of courtship or mate preference that have diverged are not clear. The male flies appeared to not recognize the heterosubspecific females as quickly as they recognized the consubspecific females. This could have resulted from a difference in the females' behavior or pheromones. Mayr (1946) presented evidence that *D. pseudoobscura* males use smell in species discrimination, so a slight change in the female *bogotana* pheromone could have produced the observed results. However, we could detect no difference in the cuticular hydrocarbons of the subspecies using gas chromatography (Noor unpublished data). Thus, a female behavioral difference seems more likely, although *D. pseudoobscura* females appear to play a passive role in courtship (Brown 1963).

Finally, the results of this study further support the idea that populations can undergo substantial genetic divergence without developing strong behavioral isolation (Coyne & Orr 1989).

ACKNOWLEDGMENT

I thank Jerry Coyne, Steve Schaeffer, Phil Service, and the National Drosophila Species Resource Center for help in obtaining fly stocks; Jerry Coyne, Norman Johnson, Shyril O'Steen, and two anonymous reviewers for helpful comments and criticisms of the manuscript; and Anne Crittenden for technical assistance and moral support. Financial support was provided by a Genetics Training Grant from the Department of Health and Human Services, a Grant-in-Aid of research from Sigma Xi, a Hinds Fund grant from the University of Chicago, and grant GM 38462 from the National Institutes of Health to Jerry Coyne.

LITERATURE CITED

- Ayala, F. J. & T. Dobzhansky. 1974. A new subspecies of *Drosophila pseudoobscura*. Pan-Pacif. Entomol., 50: 211-219.
- Brown, R. G. B. 1963. Courtship behaviour in the *Drosophila obscura* group. I.: *D. pseudoobscura*. Behaviour, 23: 61-106.
- Brown, R. G. B. 1965. Courtship behaviour in the *Drosophila obscura* group. Part II. Comparative studies. Behaviour, 25: 281-323.
- Carson, H. L., K. Y. Kaneshiro & F. C. Val. 1989. Natural hybridization between the sympatric Hawaiian species *Drosophila silvestris* and *Drosophila heteroneura*. Evolution, 43: 190-203.
- Coyne, J. A. & A. A. Felton. 1977. Genic heterogeneity at two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *D. persimilis*. Genetics, 87: 285-304.
- Coyne, J. A. & H. A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution, 43: 362-381.
- Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat., 74: 312-321.
- Dobzhansky, T., A. S. Hunter, O. Pavlovsky, B. Spassky & B. Wallace. 1963. Genetics of natural populations. XXXI. Genetics of an isolated marginal population of *Drosophila pseudoobscura*. Genetics, 48: 91-103.

- Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *Drosophila pseudoobscura* and *Drosophila persimilis*. Proc. Natl. Acad. Sci. USA, 32: 128–137.
- Prakash, S. 1972. Origin of reproductive isolation in the absence of apparent genic differentiation in a geographic isolate of *Drosophila pseudoobscura*. Genetics, 72: 143–155.
- Ryan, M. J. & W. Wilczynski. 1991. Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). Biol. J. Linn. Soc., 44: 249–271.
- Schaeffer, S. W. & E. L. Miller. 1991. Nucleotide sequence analysis of Adh genes estimates the time of geographic isolation of the Bogota population of *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. USA, 88: 6097–6101.
- Schaeffer, S. W. & E. L. Miller. 1992. Estimates of gene flow in *Drosophila pseudoobscura* determined from nucleotide sequence analysis of the alcohol dehydrogenase region. Genetics, 132: 471–480.
- Singh, R. S. 1983. Genetic differentiation for allozymes and fitness characters between mainland and Bogota populations of *Drosophila pseudoobscura*. Can. J. Genet. Cytol., 25: 590–604.
- Sokal, R. R. & F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco.
- Spieth, H. T. 1951. Mating behavior and sexual isolation in the *Drosophila virilis* species group. Behaviour, 3: 105–145.
- Tan, C. C. 1946. Genetics of sexual isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics, 31: 558–573.